addition, the frequency of tight binders in this mutagenesis approach was observed to be about 5%, whereas the frequency is approximately 3% in other selections.

REMARKS

The specification has been amended to provide a unique sequence identification number for each nucleotide sequence in the specification. The attached sequence listing has also been inserted into the application. No new matter is introduced by any of these amendments.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 22 May 2002

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Version with Markings to Show Changes Made

Replace the paragraph beginning on page 16, line 26 and ending on page 17, line 1 with the following paragraph.

FIGURE 4 is a graph illustrating a sequence alignment between a fibronectin type III protein domain and related protein domains (SEQ ID NOs. 33, 186-200).

Replace the paragraph beginning on page 18, line 11 and ending on page 18, line 16 with the following paragraph.

FIGURE 18 is a graph showing an alignment of the primary sequences of the llama V_H domain and the wild-type human ^{10}Fn3 domain. Homologous residues between the two sequences are indicated. The ^{10}Fn3 residues outside the randomized loops that were found to have mutated in approximately 45% of the selected clones are marked with arrows under the wild-type ^{10}Fn3 sequence and with the letter that identifies the selected residue (SEQ ID NOs: 201, 202).

Replace the paragraph beginning on page 19, line 5 and ending on page 19, line 6 with the following paragraph.

FIGURE 25 is a graph listing exemplary TNF-α binders (SEQ ID NOS: 33-141) according to the invention.

Replace the paragraph beginning on page 38, line 3 and ending on page 38, line 6 with the following paragraph.

Thirty-eight of the 61 clones derived from R9 and from R10 had unique amino acid sequences, a surprising diversity. The ten clones that were isolated more than once, presumably because of their superior binding to TNF-α, are listed in Table 1 (SEQ ID NOs: 141-151; full sequences in Figure 25).

Replace the paragraph beginning on page 39, line 9 and ending on page 39, line 16 with the following paragraph.

The sequences selected most frequently in the BC loop is NRSGLQS (12/61) (SEQ ID NO: 31), whereas the sequence selected most commonly in the FG loop is AQTGHHLHDK (6/61) (SEQ ID NO: 32). An NRSGLQS BC loop and an AQTGHHLHDK FG loop have not been found in the same molecule, but two clones were found which contain the most frequently isolated sequences on two of the three randomized loops. These clones, T10.06 (BC: NRSGLQS, DE: PWA; SEQ ID NO: 143) and T09.12 (DE: PWA, FG: AQTGHHLHDK; SEQ ID NO: 144), have two of the lowest four dissociation constants from TNF-α of the clones examined (Table 1).

Replace the paragraph beginning on page 45, line 4 and ending on page 45, line 11 with the following paragraph.

Twenty-two clones derived from the DNA eluted after four further rounds of selection (R14) were picked at random and found to represent 15 different loop sequences (Table 2; SEQ ID NOs: 141, 152-165; full sequences in Figure 25). The clone T10.06, isolated previously from R10 as described above, was picked eight separate times, whereas the remaining sequences, including T09.31, which had been isolated from the R9 pool, were found in one isolate each. Similar to the isolates from rounds nine and ten, the R14 clones examined showed a preference (18 of 22 clones) for the PWA/G sequence in the DE loop, and four new, non-wild-type DE sequences were revealed.

Replace the paragraph beginning on page 45, line 20 and ending on page 46, line 14 with the following paragraph.

As discussed above, the selections described herein may also be combined with mutagenesis after all or a subset of the selection steps to further increase library diversity. In one parallel selection strategy, error-prone PCR was incorporated into the amplification of DNA between rounds (Cadwell and Joyce, PCR Methods Appl 2:28 (1992)). This technique was carried out beginning with the diverse DNA pool eluted after R8 above. This pool was amplified using error-prone PCR, with the pool divided into seven equal parts and mutagenized at the target frequency of 0.8%, 1.6%, 2.4%, 3.2%, 4.0%, 4.8%, and 5.6%. The seven PCR reactions were combined, and cDNA/RNA-protein fusion was made from the mixture and subjected to a round of

selection in solution. Before the second mutagenic round, M10, error-prone PCR was performed in three separate reactions, at 0.8%, 1.6%, and 2.4%. The two remaining rounds, M11 and M12, were performed using standard Taq PCR. Except for mutagenesis, the selection conditions for M9-M12 were the same as for R11-R14. The twenty M12 clones tested showed tighter binding to TNF-α than the clones selected using the two earlier selection protocols (Table 3; SEQ ID NOs: 141, 166-185; full sequences in Figure 25); the tightest binding of TNF-α was seen in M12.04, and had the observed K_d of 20 pM. These results demonstrated that low-level, random mutagenesis late in a selection can improve both the binding affinity of selected antibody mimics (20 pM vs. 90 pM) and the speed with which they can be selected (12 rounds vs. 14 rounds). In addition, the frequency of tight binders in this mutagenesis approach was observed to be about 5%, whereas the frequency is approximately 3% in other selections.